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(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF INFECTIOUS DISEASES

(57) Abstract

The synergistic combination of certain bridged diphenyl compounds with oxidant agents for the treatment of infectious diseases is disclosed. These diphenyl compounds are exemplified by 2,3,4,3',4',5'-hexahydroxybenzophenone.

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PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF INFECTIOUS DISEASES

TECHNICAL FIELD

This invention pertains to therapeutic compositions for the treatment of infectious diseases, including parasitic diseases such as malaria. More specifically, the invention concerns therapeutic compositions that act synergistically with oxidant agents which induce "oxidant stress."

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BACKGROUND OF THE INVENTION

Protozoan parasites cause diseases such as malaria, trypanosomiasis, Chagas' disease, leishmaniasis, giardiasis, and amoebiasis. These and other protozoan parasite diseases have historically occurred in tropical and sub-tropical areas where they cause widespread damage to human populations. Although they receive little attention in the Western world, protozoan diseases affect more people worldwide than diseases brought on by any other biological cause (Heyneman, 1988).

The global importance of just one parasitic protozoan disease, malaria, can be appreciated by considering that historically, almost half of the human race has died from malaria. Today, malaria remains the most destructive single infectious disease in the developing world. It is responsible for more human energy loss, more debilitation, more loss of work capacity, and more economic damage than any other human ailment facing the world today (Heyneman, 1988). The World Health Organization estimates that 1 to 2 million deaths are caused by malaria each year in Africa alone; most of these are children under the age of five (World Health Organization, 1991). In addition, over 300 million people worldwide are believed to be chronically-infected, and each year nearly one third of these individuals will suffer acute manifestations of the disease.

Today, the pathologic capacity of protozoa is being increasingly demonstrated in the Western world among the victims of AIDS (Acquired Immunodeficiency Syndrome). AIDS depletes the immune system of affected individuals; this allows opportunistic agents which would be defeated by an active immune system to infect AIDS patients. Several protozoans have emerged as important opportunistic infections in AIDS patients including *Cryptosporidium parvum, Entamoeba histolytica, Giardia lamblia, Pneumocystis carinii* (which may be a fungal or protozoal pathogen), and *Toxoplasmosis gondii*.

Despite the prevalence and significance of protozoan infections, therapy for these diseases is generally poor or in need of improvement. Many chemotherapeutic agents used to treat protozoan infections are non-specific cytotoxins that are highly toxic and cause severe side effects in patients. However, these drugs are used because there are no better alternatives. For example, giardiasis and amoebiasis are treated using metronidazole (a nitroimidazole), but the use of this drug is clouded by its mutagenic potential (Campbell, 1986) and its adverse interaction with alcohol. For trypanosomiasis and leishmaniasis standard therapies (suramin, melarsoprol, and pentavalent antimonials) are dangerously toxic, occasionally fatal, and often ineffective (Mebrahtu, 1989; Grogl et al., 1992). Other drugs are becoming ineffective due to emerging resistance. In the case of malaria, effective therapy has previously been provided by chloroquine but its efficacy is now threatened by the rapid emergence of drug resistant strains of *Plasmodium falciparum*, the causative agent for the most severe, often fatal, form of the disease (Cowman, 1990). Other protozoal infections such as cryptosporidiosis or Chagas' disease have no proven curative agent.

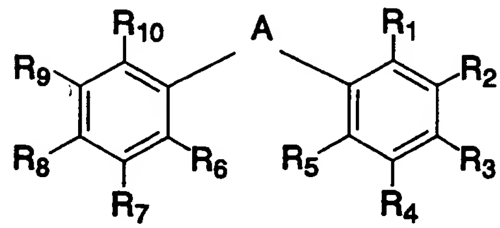
A group of therapeutic agents collectively referred to as oxidant drugs holds the promise for effective treatment of multi-drug resistant *Plasmodium* parasites (Vennerstrom et al., 1988). These drugs cause enhanced production of oxygen radicals inside parasitized erythrocytes (Golenser et al., 1991) or act to render parasites (or their host cells) more susceptible to attack by oxygen radicals. The anti-malarial oxidant agents are structurally diverse and include seemingly unrelated compounds such as methylene blue, ascorbic acid, Atovaquone® (a hydroxynapthoquinone), tetracyclines, ketoconazole, artemisinin, and the active metabolites of primaquine, (Vennerstrom et al., 1988). Despite their structural diversity these agents share the ability to produce or liberate free radical oxygen species, or to render the parasites or their host cells more susceptible to oxygen radical attack. Certain of these compounds are currently in use as anti-malarial agents (e.g., artemisinin, tetracycline and primaquine) or are in the later stages of clinical development (e.g., Atovaquone) (Hong et al., 1994; Hudson et al., 1991). However, because Plasmodia are known to develop resistance to singly active drugs when used alone, there is a need to develop new drugs which act in combination and in synergy with these anti-malarials.

The present invention is directed to new antiparasitic agents, and in particular to new antiparasitic agents that act synergistically with oxidant drugs. A particular object of the present invention is the development of new drugs that act synergistically with oxidant anti-malarial drugs.

SUMMARY OF THE INVENTION

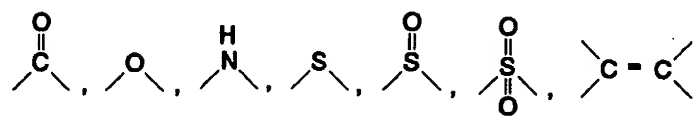
The present invention is based on the discovery that certain compounds, based on formula 1 below, act synergistically with oxidant drugs.

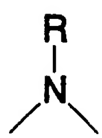
Formula 1:



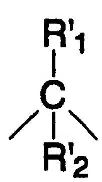
wherein:

A is selected from the group consisting of





wherein R is H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine and



wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine;

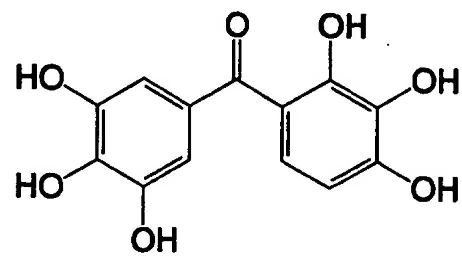
wherein R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, $OCOCH_3$ ("OAc"), OCH_3 ("OMe"), NH_2, SO_3^-, N_3, alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl;

and wherein at least one f R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H. In the substituents provided for formula 1 compounds in the foregoing

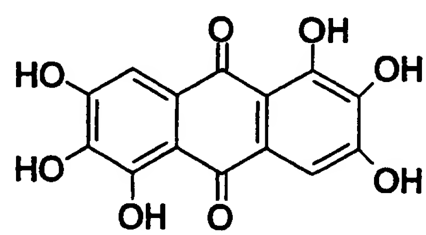
description, alkyl in any of the named substituents is pref rably a C_{1-20} alkyl (including straight chain, branched and cyclic alkyls), more preferably a C_{1-10} alkyl and most preferably a C_{1-5} alkyl.

By way of illustration, the diphenyl compound 2,3,3',4,4',5'-hexahydroxybenzophenone (also known as exifone or adlone), shown below as formula 2, acts synergistically with the oxidant drug rufigallol, enhancing anti-malarial activity 350 fold. Rufigallol (1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone) is shown below as formula 3. The anti-malarial activity of rufigallol is also demonstrated for the first time in this invention.

Formula 2: (exifone)



Formula 3: (rufigallol)



The invention that results from this discovery encompasses new drug combinations for the treatment of malaria and other infectious diseases. In one embodiment, the invention comprises the combination of a compound according to formula 1 with an oxidant agent, wherein the oxidant agent is a drug useful in treating an infectious disease. For example, the oxidant agent may be rufigallol and the formula I compound may be 2,3,4,3',4',5' hexahydroxybenzophenone (such that A is carbonyl, R_1-R_3 and R_7-R_9 are hydroxy and R_4-R_6 and R_{10} are hydrogen) (exifone). The combination of the oxidant drug with the formula 1 compound produces a synergistic effect against the infectious agent, allowing lower doses of the oxidant drug to be employed while maintaining or enhancing efficacy, and reducing the likelihood for development of resistance to the oxidant drug.

Notably, the oxidant agent used in combination with the formula 1 compound need not be a conventional drug used against infectious diseases because formula 1 compounds are

effective against infectious agents when activated by a wide range of oxidant agents, including ascorbic acid (vitamin C). Activation may also be achieved by the use of gamma irradiation, an oxidant agent known to kill parasitized cells as a result of oxygen radical production. Accordingly, in another embodiment, the invention comprises the combination of the compound according to formula 1 with an oxidant compound, such as ascorbic acid. In a particular example, the oxidant agent may be ascorbic acid (vitamin C) and the formula I compound may be 2,3,4,2',3',4' hexahydroxybenzophenone (isofone).

Another aspect of the invention is the use of rufigallol (1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone) and certain analogs of this compound as an anti-malarial drug.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphical representation of the anti-malarial activity of rufigallol against the mefloquine-resistant D6 strain of *P. falciparum*.

Fig. 2 is an isobolar analysis of the synergistic drug combination of rufigallol and exifone.

Fig. 3 is an isobolar analysis of the synergistic combination of ascorbic acid and exifone.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions:

Oxygen radicals: as used herein, the term oxygen radicals refers to free radicals containing oxygen, including superoxide radicals and hydroxy radicals.

Oxidant compound: an oxidant compound is one that produces oxygen radicals, particularly when heated or when reacted with other compounds. In particular, the oxidant compounds described herein produce oxygen radicals in or on the body of an animal when administered to the animal. An example of an oxidant compound is ascorbic acid, which yields oxygen radicals in the presence of oxygen and a transition metal.

Oxidant drug: an oxidant drug is a pharmaceutical or therapeutic substance that is used to treat a particular condition in animals, such as an infectious disease, wherein the drug either causes enhanced production of oxygen radicals inside cells, or renders cells more susceptible to attack by oxygen radicals, or is capable of oxidizing another compound. An example of an oxidant drug is tetracycline.

Oxidant agent: a term encompassing oxidant compounds, oxidant drugs and other agents having the ability to produce or liberate free radical oxygen species or to render parasites or their host cells more susceptible to oxygen radical attack, or is capable f oxidizing another compound. Ascorbic acid, hydrogen peroxide and tetracycline are oxidant agents, as is gamma radiation. Indeed, the "respiratory burst" of a macrophage or granulocyte is also an oxidant.

2. Materials and Methods

a. Chemicals and Reagents

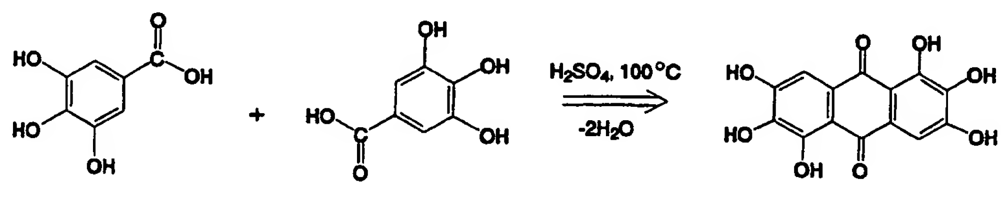
Anthraquinones

The following anthraquinones were purchased from the Aldrich Chemical Company (Milwaukee, Wisconsin): 1,5-dihydroxy-9,10-anthraquinone; 2,6-dihydroxy-9,10-anthraquinone; and 1,2,4-trihydroxy-9,10-anthraquinone.

9,10-anthraquinone was purchased from Janssen Chimica (Belgium).

2,3,6,7-tetrahydroxy-9,10-anthraquinone was synthesized according to the method of Boldt, 1967.

Rufigallol (1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone) was synthesized by dehydration of gallic acid according to the method of Haworth and Grimshaw, 1956 as depicted in the following scheme:



Gallic acid

Ruffigallol

The methyl ethers of rufigallol and other selected anthraquinones were synthesized from the free hydroxy compounds by refluxing in the presence of potassium carbonate in acetone with an excess of dimethyl sulfate. Rufigallol-hexamethyl ether was converted to the dibromide form with trifluoroacetyl-hypobromite. Hexaacetyl rufigallol (1,2,3,5,6,7 hexaacetoxy-9,10-anthraquinone) was obtained by treatment of rufigallol with acetic anhydride.

Octahydroxy-9,10-anthraquinone was synthesized according to Georgievics, 1911.

Benzophenones

All of the benzophenones tested except for exifone and isofone were purchased from Aldrich Chemical Company (Milwaukee, WI); 2,3',4,4',6-pentahydroxybenzophenone (also known as maclurin) being obtained from the Sigma-Aldrich rare chemical collection.

Exifone (2,3,4,3',4',5'-hexahydroxybenzophenone) was synthesized by a procedure based on the method reported by Bleuler and Perkins, 1916. A mixture of gallic acid (5.00g, 29.4mmol), pyrogallol (3.72 g, 29.4mmol) and anhydrous zinc chloride (12.16g, 89mmol) was heated in an Erlenmeyer flask with stirring. Within 45 minutes, the flask, which was placed in a sand bath on a hot plate, was brought to a temperature of 120°C. After this period, clumps

formed in the flask. The temperature of the sand bath was gradually raised to 150°C and this temperature was maintained for an additional 30 minutes. During this elevation in temperature, at ca. 150°C, a dark syrup formed which could be stirred. After allowing the reaction mixture to cool to room temperature, 100ml of distilled water was added and the resulting solution was heated until ebullition. Upon cooling, the dark clear solution developed a thick crystalline precipitate. The material was re-crystallized three times from 400ml of water. After air-drying, 0.765 gm of a dirty-yellow lustrous mass was obtained. The final product appeared as a mass of small yellow crystals with an almond scent. Further characterization by ultraviolet/visible spectrophotometry, proton nuclear magnetic resonance, elemental analysis, gas chromatography/mass spectrometry all confirmed identity with literature values for exifone. Exifone is also available commercially from Pfaltz and Bauer Inc., Waterbury, CT, and Acros Organics, Pittsburgh, PA. Exifone and isofone may also be synthesized by the methods described in Grover et al. (1956).

Other Materials

RPMI-1640 culture medium and gentamicin from Gibco (Grand Island, NY) were employed. Radiolabelled [^{3}H]-ethanolamine was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

b. Culture of Plasmodium falciparum and drug testing

The D6 strain of *P. falciparum* was cultured in Group A$^+$ human erythrocytes and suspended at a 3.3% hematocrit in RPMI-1640 (containing 4g/L glucose, 50mg/L gentamicin and 10% group A$^+$ human serum), buffered with 25mM HEPES and 25 mM $NaHCO_3$ (Larrey, 1989). Cultures were maintained at 37°C in a gas mixture of 3% oxygen, 5% CO_2, and 92% nitrogen. *In vitro* anti-malarial activity of test compounds was measured by the [^{3}H]-ethanolamine incorporation method as described in Elabbadi et al., 1992, with minor modifications.

Briefly, experiments were conducted in 96 well plates in a total volume of 200μl at a final red blood cell concentration of 3.3% (v/v). An initial parasitemia of 0.2 to 0.5% was attained by addition of normal uninfected red cells. Radiolabelled ethanolamine was added after 48 hours of incubation and the experiment was terminated after 72 hours by collecting the cells onto glass fiber filters with an automated multiwell harvester.

Stock solutions of exifone and the various benzophenones were made by dissolving each compound into dimethylsulfoxide (DMSO) at 10mM. (Ethanol, dimethylformamide, or propylene glycol could be substituted for DMSO without altering any of the results). All dilutions were made into medium containing 10% human serum. The final concentration of DMSO never exceeded 1% (v/v) which control experiments demonstrated was not sufficient to influence the rate of radiolabel incorporation into parasitized erythrocytes. The concentration of drug giving 50% inhibition of label incorporation (IC_{50}) relative to control (i.e., drug-free) conditions was calculated from the dose-response curve.

3. Examples of oxidant drugs

A. *Oxidant drugs effective against malaria*

The oxidant sensitivity of malaria infected erythrocytes has been recognized for some time. This sensitivity likely arises from the generation of oxygen radicals by the parasite itself and from a weakening of the oxidant defense mechanisms of the host erythrocyte by the parasite. Oxidant drugs such as the hydroxynaphthoquinones exploit this weakness by causing the formation of toxic oxygen radicals (and hence additional oxidant "stress") inside parasitized cells.

As discussed above, oxidant anti-malarial drugs are structurally diverse but share the ability to liberate or cause the enhanced production of intracellular free radical oxygen species or to render parasitized cells more susceptible to oxygen radicals. Putative oxidant anti-malarial drugs include methylene blue, ascorbic acid, tetracycline, ketoconazole and the active metabolite of primaquine. One group of oxidant anti-malarial drugs that has received considerable recent attention is the hydroxynaphthoquinones.

i. Hydroxynaphthoquinones

The anti-malarial activity of the hydroxynaphthoquinones has been recognized since the 1940's (Fieser et al., 1948). Work on these compounds continued only sporadically (Fieser et al., 1967a-c) probably because of the successful development of aminoquinolines such as chloroquine and mepacrine. However, because of the emergence of resistance among malaria parasites to the aminoquinolines, there is now renewed interest in development of an anti-malarial naphthoquinone.

Recent research in this area has resulted in the production and testing of a new hydroxynaphthoquinone, termed Atovaquone®. This drug appears to block dihydro-orotate dehydrogenase, effectively preventing pyrimidine biosynthesis in the malarial parasite which does not possess the biochemical machinery for salvaging pyrimidines (Sherman, 1979). However, another site of action of Atovaquone® appears to be the diversion or blockage of electrons which normally flow from dihydro-orotate dehydrogenase through a ubiquinone-linked electron transport system (Fry et al., 1992). Thus, Atovaquone® may act as a catalytic oxidizing agent capable of undergoing cyclic one-electron oxidation-reduction reactions. Such futile "redox-cycling" would lead to the catalytic reduction of oxygen to superoxide ($^{\bullet}O_2$-) at the expense of reducing equivalents such as NAD(P)H (Vennerstrom et al., 1988). In the presence of soluble iron, the reactive superoxide radical anion could be converted to the highly-reactive hydroxyl radical ($^{\bullet}OH$) via the metal-catalyzed Fenton reaction (Golenser et al., 1991). Both superoxide and hydroxyl radicals have the capacity to modify proteins, lipids, and nucleic acids of the parasite and the red cell host, thereby killing the parasite.

ii. Anthraquinone derivatives

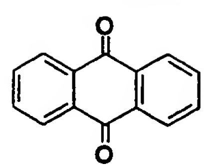
One aspect of the present invention is the discovery that certain anthraquinone derivatives, and in particular 1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone, also known as rufigallol, have potent anti-malarial activity.

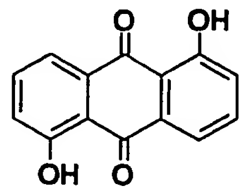
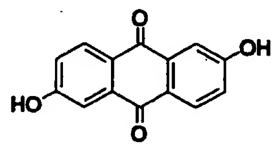
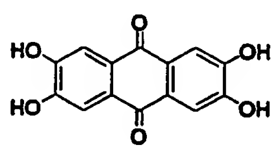
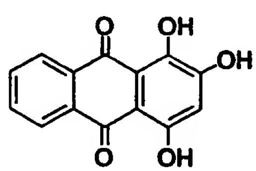
Anthraquinone derivatives were synthesized and tested for their ability to inhibit the growth of the malarial parasite *P. falciparum* by the methods described above. As shown in Table 1, most of these compounds are relatively poor anti-malarial agents, requiring concentrations of greater than 1,000nM to achieve demonstrable effects. However, rufigallol is noted to be an extremely potent anti-malarial agent. As shown in Table 1, the concentration of rufigallol required to inhibit the growth of *P. falciparum* by 50% (i.e., to produce an IC_{50}) is approximately 35nM. Chloroquine, a standard anti-malarial agent has an IC_{50} under these test conditions of 20nM. Rufigallol is equally effective against the multi-drug resistant form of *P. falciparum* strain W2 (data not shown).

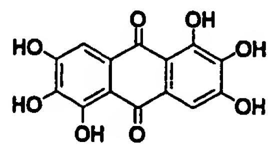
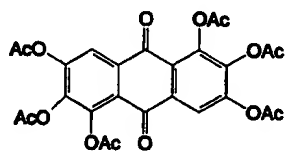
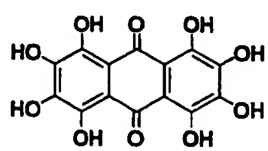
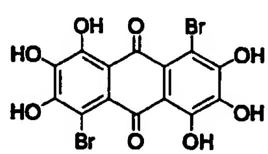
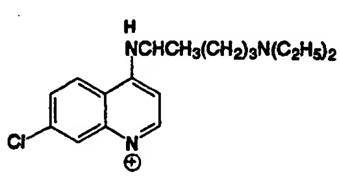
The tetrahydroxy- and octahydroxy-derivatives of rufigallol are moderately effective, exhibiting IC_{50} values of 300nM and 800nM, respectively. The hexacetoxy derivative of rufigallol is also moderately effective, having an IC_{50} value of 350nM. In contrast, the hexamethyl ether form is significantly less potent (IC_{50} of >5000nM, data not shown). The insertion of two bromine atoms onto the rufigallol structure to form 1,5-dibromohexahydroxy-9,10-anthraquinone yielded a compound essentially devoid of anti-malarial activity.

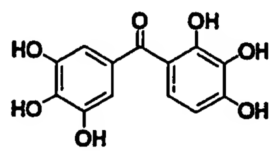
Exifone, represented in Table 1 as a rufigallol analog lacking one of the keto moieties and thus the internal aromatic ring, exhibits only weak antiplasmodial activity (IC_{50} of approximately 4100nM).

Table 1

Chemical Structure	Name(s)	IC_{50},nM
	9,10-anthraquinone	5000

	1,5-dihydroxy-9,10-anthraquinone (anthrarufin)	>1000
	2,6-dihydroxy-9,10-anthraquinone (anthraflavic acid)	>1000
	2,3,6,7-tetrahydroxy-9,10-anthraquinone	300
	1,2,4-trihydroxy-9,10-anthraquinone (purpurin)	>1000

	1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone (rufigallol)	35
	1,2,3,5,6,7 hexaacetoxy-9,10-anthraquinone (hexacetoxy rufigallol)	350
	octahydroxy-9,10-anthraquinone	800
	1,5-dibromo-hexahydroxy-9,10-anthraquinone	5000
	Chloroquine, a standard antimalarial agent	20

	2,3,4,3',5',5'-hexahydroxybenzo-phenone (exifone, adlone)	4100

iii. Rufigallol as an anti-malarial agent

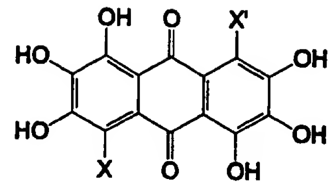
The anti-malarial activity of rufigallol against the D6 strain of *P. falciparum* is shown in Fig. 1. As noted above, rufigallol exhibits an IC_{50} for *P. falciparum* infected erythrocytes of roughly 35nM.

Toxicity tests were carried out to determine the effects of rufigallol on the growth and differentiation of normal human bone marrow stem cells *in vitro*. For these experiments, human granulocyte-macrophage progenitor cells were grown in agar with a standard source of colony-stimulating activity (Burgess et al., 1986). The results are shown below in Table 2, expressed as the average colony count of colony forming units of granulocyte/macrophage (CFU-GM) after incubation at 37°C for 10 days in a humidified 5% CO_2 incubator with rufigallol. Deleterious effects were exerted by rufigallol at concentrations above 10μM, at which concentration approximately 35% suppression of colony growth was noted. In parallel experiments conducted under the same test conditions, chloroquine produced a modest degree of suppression at a concentration of 10μM.

Table 2. Toxicity of rufigallol toward human bone marrow stem cells (CFU-GM) *in vitro*.

Culture Conditions	Average colony count, CFU-GM	
[Drug], μM	Rufigallol	Chloroquine
Control (no additions)	56 ± 11	56 ± 11
0.1	61 ± 11	56 ± 4
1.0	57 ± 8	54 ± 5
5.0	47 ± 6	54 ± 5
10.0	36 ± 9	48 ± 3
100.00	5 ± 2	5 ± 3

One aspect of the present invention is thus the discovery that rufigallol and certain analogs of this compound are effective anti-malarial agents. These new anti-malarial agents may be represented by the formula:



wherein X and X' are the same or different and are independently selected from the group consisting of alkyl, haloalkyl, diaminoalkyl, haloaminoalkyl, amino, nitro and azido. In this formula, an "alkyl" is preferably a C_{1-20} straight chain, branched or cyclic alkyl, and more preferably a C_{1-5} alkyl.

A patient suffering from malaria can be treated by administration of rufigallol or an analog of rufigallol according to the formula above. The compound may be administered by any known administration technique, for example orally (such as in the form of a pill) or by intramuscular or intravascular injection. In preferred embodiments, the compound is combined with a pharmaceutically acceptable excipient prior to administration. A patient is preferably given a dose of rufigallol or a rufigallol analog, as discussed above, in the range of 10-2,000 mg/kg/day. The dosage will vary depending on known factors, such as the age and condition of the patient. It will be appreciated that a therapeutically effective dosage is one which preferably results in complete remission of the malaria. However, a therapeutically effective dosage also encompasses those dosages which slow or limit the further development of the malarial infection.

While not wishing to be bound by speculation, the inventors propose that the mechanism of action of hydroxyanthraquinones such as rufigallol is futile redox cycling leading to the catalytic reduction of oxygen to superoxide and, ultimately to the formation of hydroxyl radicals.

B. *Oxidant drugs effective against other infectious diseases*

Numerous drugs used to treat infectious diseases act through an oxidant mechanism or possess an independent oxidant activity. Such drugs include: difluoromethylornithine (DFMO), melarsoprol and stibogluconate (for the treatment of leishmaniasis and trypanosamiasis), metronidazole (for giardiasis and ameobiasis among others), buparvaquone (for leishmaniasis) artemisinin (for malaria), hydrogen peroxide and benzoyl peroxide (for bacterial infections), and ascaridole and bischloroethyl nitrosurea (for helminth infections). Other drugs, such as doxorubicin for cancer treatment, also have oxidant activity.

4. <u>Examples f Oxidant Compounds</u>

Apart from drugs that have oxidant activity, many other compounds are known to possess oxidant activity. Such compounds include: isoascorbic acid, quercetin, and alloxan.

5. P tentiation of oxidant drugs by formula 1 compounds

The ability of formula 1 compounds to potentiate the efficacy of oxidant drugs is illustrated by the combination of the formula 1 compound exifone with the oxidant anti-malarial drug rufigallol.

The potentiation of rufigallol by exifone was demonstrated by combining the two compounds in varying concentrations and utilizing the incorporation of radioactive ethanolamine into parasite lipids as described in Materials and Methods as an objective measure of parasite growth. These experiments were initiated with synchronized mature trophozoites. Mature trophozoites were produced by incubating synchronized ring forms (selected by the sorbitol lysis technique described by Lambros et al., (1979)) for 16-24 hours. Standard isobolar analysis was employed for evaluating synergism between the drug combinations.

Table 3 shows the drug concentrations of rufigallol and exifone required to achieve an IC_{50} (alone or in combination). The average IC_{50} values for rufigallol alone and exifone alone collected from 8 independent experiments were 75nM and 3.75μM, respectively. In combination however, striking synergy was observed between rufigallol and exifone. This synergy is shown in the graphical representation of these experiments shown in Fig. 2. The striking synergy is indicated in Fig. 2 by the downward deviation of the values relative to the theoretical line of addition. For example, the combination of 1nM rufigallol with 10nM exifone delivered the same growth inhibitory effect as either drug alone at their respective IC_{50} dose.

A geometric calculation of the degree of potentiation (using the isobole shown in Fig. 2) indicated a value of approximately 350-fold.

Table 3

Rufigallol IC_{50} conc., nM	Exifone IC_{50} conc., μM
0.000	3.75
0.1	1.25
1	0.01
10	0.01
10	0.001
75	0.000

As illustrated by this data, an aspect of the present invention is the discovery that oxidant drugs such as those discussed above act synergistically with formula 1 compounds such as 2,3,4,3',4',5'-hexahydroxybenzophenone (exifone). In light of the weak activity of exifone against *Plasmodium falciparum* in vitro, the discovery of potent synergism between exifone and oxidant drugs, such as rufigallol, is of great surprise.

A second formula 1 compound, 2,3,4,2',3',4'- hexahydroxybenzophenone, "isofone," was also tested for its ability to potentiate the activity of rufigallol. As illustrated in

Table 4, this compound potentiated the activity of rufigallol in a manner similar t that observed with exifone.

Exifone was also tested in combination with three other oxidant drugs using the same assay method and potentiation was noted in each case: artemisinin (4-6-fold potentiation); doxorubicin (5-fold potentiation); and metronidazole (12-15-fold potentiation).

Other benzophenones were tested for ability to potentiate rufigallol using the method described above for rufigallol and exifone. The results are presented in Table 4. As shown, the ability of formula 1 compounds to greatly enhance the anti-malarial activity of rufigallol is not a characteristic possessed by all of the benzophenones tested.

Table 4. Comparison of the ability of benzophenones and related compounds to augment the activity of rufigallol.

Drug	IC_{50}	Estimated degree of potentiation
benzophenone	-100μM	none
2,2'-dihydroxybenzophenone	2.5μM	none
3,3'-dihydroxybenzophenone	10μM	none
4,4'-dihydroxybenzophenone	>50μM	none
2,4-dihydroxybenzophenone	75μM	none, additive
3,4,3',4'-tetrahydroxybenzophenone	10μM	none
2,2'4,4'-tetrahydroxybenzophenone	37.5μM	none, additive
2,3,4,3',4',5'-hexahydroxybenzophenone (exifone)	3.75μM	350-fold
2,3,4,2',3',4'-hexahydroxybenzophenone (isofone)	2.5μM	350-fold
2,3',4,4',6-pentahydroxybenzophenone	50μM	2-fold
2,3,4,3',4',5'-hexamethoxybenzophenone	>100μM	none
2,2'-dipyridylketone	>50μM	none

6. Activation of formula 1 compounds by oxidant compounds

Activation of formula 1 compounds is shown above to occur in the presence of oxidant drugs, such as rufigallol. The oxidant activation of formula 1 compounds may also be achieved through the action of other oxidant compounds. By way of illustration, data showing the activation of formula 1 compounds by ascorbic acid (vitamin C) is presented below.

The ability of ascorbic acid to act synergistically with exifone to inhibit the growth of *P. falciparum* was ascertained using the methods described above. A typical result is sh wn in Fig. 3; the two compounds show potent synergism. A geometric calculation of the degree of potentiation indicated a value f approximately 100-fold.

While this result may seem surprising since ascorbic acid is considered an antioxidant in humans, Marva et al. (1992) have demonstrated the pro-oxidant activity of ascorbic acid on infected erythrocytes. This activity probably results from an intra-erythrocytic Fenton reaction occurring in the acidic food vacuole of the parasite wherein iron and heme are liberated as hemoglobin is progressively digested by the growing plasmodium. According to Fenton chemistry, ascorbate serves to reduce iron (Fe^{+3}) in the presence of oxygen and the resulting ferrous iron (Fe^{+2}) catalyzes the conversion of superoxide into the highly reactive hydroxyl radical (Halliwell et al., 1988).

7. <u>The effect of oxygen tension on formula 1 compounds</u>

Oxygen dependence has been demonstrated for the anti-malarial effects of a number of compounds including tetracyclines, artemisinin and imidazoles. As indicated in Table 5 below, the potency of exifone alone is increased by six fold when oxygen tension was increased from 5% to 15%. Significantly, the impact of higher oxygen tension was even greater when ascorbate was added to exifone; the IC_{50} value for exifone decreased to below 0.1μM.

Table 5

	Exifone IC_{50}, μM^a	
Conditions	Control (no additions)	+ ascorbate (100μM)
Gas Mixture ($5\%\ O_2/5\%\ CO_2/90\%N_2$)	4.4 ± 2.4	0.9 ± 0.4
Candle jar (~15% O_2)	0.72 ± 0.3	0.095 ± 0.05

[a]IC_{50} values represent the mean ± standard deviation from at least 5 separate experiments each performed in duplicate.

It is known that the anti-malarial activity of tetracycline is enhanced by higher oxygen tension. To investigate the effect of oxygen tension on the combination of tetracycline and formula 1 compounds, similar experiments to those described above were performed for tetracycline in combination with exifone. Under standard assay conditions, tetracycline exhibits an IC_{50} of only 10-20μM for strain D6 with an oxygen tension of 5%. When the oxygen tension is raised to 15%, the IC_{50} decreases to 0.6μM. When tetracycline and exifone are combined in an oxygen tension of 15%, a synergy of 2-3 fold is detected.

8. <u>Toxicity studies</u>

The toxicity of exifone, alone and in combination with rufigallol, toward mammalian cell lines in vitro was determined. Human K562 and HL-60 cells were cultured as described previously (Collins et al., 1977; Breitman et al., 1980, 1984). The cells were seeded at 2 x 10^5 cells per ml and incubated in the presence or absence of the drug(s). After a 5 day period, cell density was determined using a hemacytometer and cell viability was assessed by monitoring for trypan blue exclusion.

As shown in tables 6 and 7, significant deleterious effects were exerted by exifone only at concentrations at and above 10μM. The combination of exifone and rufigallol was no more toxic in these mammalian cell systems than the sum of their respective inhibitory activities. Thus, while malaria parasites are extremely susceptible to the inhibitory effects of exifone and rufigallol in combination, mammalian cells appear to be much less affected. This indicates that a therapeutic drug combination of exifone and an oxidant drug may exhibit a high therapeutic index.

Table 6. Effect of exifone on the proliferation of K562 and HL-60 cells *in vitro*.

Conditions	K562 (% of control)	HL-60 (% of control)
Control (no drug added)	100%	100%
10μM Exifone	47.6	0
5μM	68.8	56.9
1μM	96.5	76.9
0.1μM	98.8	90.0
0.01μM	85.3	106.1

Table 7. Effect of exifone and rufigallol in combination on the proliferation of K562 and HL-60 cells *in vitro*.*

Conditions	K652	HL-60
Control (no drug added)	100%	100%
10 μg/ml rufigallol + 35 μg/ml exifone (33μM rufigallol w/126 μM exifone)	6	0
2 μg/ml rufigallol + 7 μg/ml exifone	32.5	17
1 μg/ml rufigallol + 3.5 μg/ml exifone	72.4	67.5
0.1 μg/ml rufigallol + 0.35 μg/ml exifone	88.4	68
0.01 μg/ml rufigallol + 0.035 μg/ml exifone	115.3	100.1

* Drugs tested at 3.5 to 1 ratio (n=1, in duplicate), exifone to rufigallol, respectively. Isobolar analysis suggests that the most efficient drug ratio may be closer to 10 or 20 to 1.

Exifone was first synthesized and patented in the late 19th Century (German Patent No. 49149). Chemical synthesis of exifone was first described in the mainstream literature by Bleuler and Perkins (1916). In recent years, exifone has been tested for use in treatment of cognitive decline associated with age in geriatric adults and patients with Parkinson's disease

(Porsolt et al., 1987; Allain et al., 1988; Descombe et al., 1989; Kai et al., 1990). Exifone was shown to improve memory function without producing a spontaneous effect on motor activity (Kai et al., 1990; Porsolt et al., 1987, 1988).

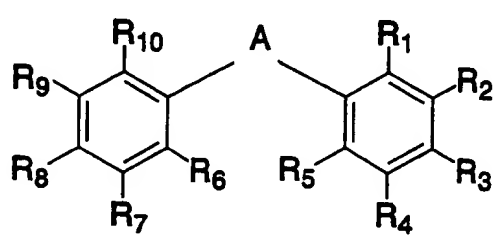
As part of these experiments it was noted that an oral dose of 1024 mg/kg exifone did not produce measurable toxicity in geriatric patients. However, continued administration of high doses of the drug to elderly patients occasionally produced some liver damage in some patients, but this damage was reversed when treatment was discontinued (Chichmanian et al., 1989; Gendreau et al., 1989; Grange et al., 1989; Larrey, 1989; Larrey et al., 1989; Pariente and Kapfer, 1989; Ouzan et al., 1990). The incidence of detectable liver damage was reported to be in the range of 1/15,000 patients (Larrey, 1989).

As discussed above, the present invention encompasses compositions comprising a formula 1 compound and an oxidant compound. The invention will be better understood by reference to the following examples, which are intended to merely illustrate the best mode now known for practicing the invention. The scope of the invention is not to be considered limited thereto, however.

EXAMPLES

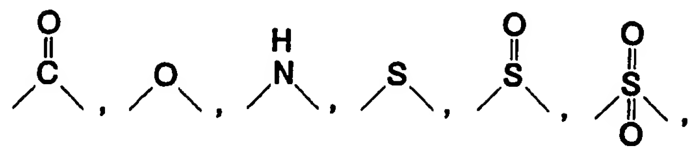
EXAMPLE 1: Scope of formula 1 compounds

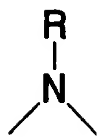
The present invention has been exemplified above by the formula 1 compound exifone. However, it will be appreciated that the scope of this invention extends generally to compounds of the formula:



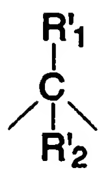
wherein:

A is selected from the group consisting of





wherein R is OH, alkyl, haloalkyl, alkylamine, or haloalkylamine and



wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine;

(that is, A can be carbonyl, oxygen, nitrogen (substituted or unsubstituted), sulphur (substituted or unsubstituted) or carbon (substituted or unsubstituted)); and

R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, OAc, OMe, NH_2, SO_3-, N_3 alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl; and

at least one of R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H.

EXAMPLE 2: Scope of oxidant agents and methods for determining oxidant activity

In part, the present invention lies in the combination of a formula 1 compound with an oxidant agent. For the purposes of the present invention, any oxidant agent may be used to activate the formula 1 compound. Where the combination of a formula 1 compound and an oxidant agent is administered to a patient suffering from an infectious disease, a preferred embodiment will be the combination of a formula 1 compound together with a known oxidant drug that is conventionally used to treat the disease, such as Atovaquone®.

Oxidant drugs either directly or indirectly cause enhanced formation of oxygen radicals in living systems. The determination of whether a drug or other compound has oxidant activity may be made by ascertaining whether the biological activity of the drug or compound can be neutralized by an anti-oxidant agent (e.g., α-tocopherol or dithiothreitol). A number of other methods of evaluating the ability of a drug to induce the formation of oxygen radicals in living cells are also known. Any of these known methods may be utilized to determine whether a particular drug or compound has oxidant activity, such that it may be used in combination with formula 1 compounds in practicing the present invention.

Monitoring for aromatic hydroxylation of salicylic acid by HPLC

Marva et al. (1992) describe a method of determining whether a drug or other compound has oxidant activity by exposing cells to salicylic acid (ortho-hydroxybenzoic acid) in the presence of the suspected oxidant drug and following the conversion of salicylic acid to the corresponding 2, 3 and 2,5-dihydroxy-benzoic acid by high performance liquid chromatography (HPLC). Typically, living cells produce a background level of oxygen radicals resulting in the detection of small quantities of the two dihydroxy compounds. An oxidant drug therefore can be expected to increase the rate of formation of hydroxyl radicals leading to a corresponding increase in the levels of the 2,5 and 2,3-dihydroxy salicylic acid derivatives.

The method is performed by the addition of 10mM salicylic acid to living cells in the presence or absence of a suspected "oxidant drug" under conditions in which the drug exerts growth inhibitory activity. At various times of incubation, suspensions of cells are centrifuged and the resulting pellets and supernatant are separated. Both fractions are treated with an equal volume of trichloroacetic acid (TCA) to precipitate macromolecules which are separated from the supernatant by centrifugation at 12,000g for 10 min. The supernatant, containing salicylic acid and its dihydroxy derivatives, is dried in vacuo in a vacuum centrifuge. The dried residue is dissolved in water and applied to an HPLC. A Lichrospher 100 (reverse phase, C_{18}, RP-18, 5μm particle size) is used for separation of salicylic acid and its hydroxylation products. The mobile phase contains 0.03M citric acid, 0.03M acetic acid, 1% methanol (vol), and 0.28 g/l sodium azide (adjusted to pH 3.6). The flow rate is 1ml/min. The biological samples are compared to standard curves of 2,5-dihydroxy and 2,3-dihydroxybenzoic acid using either an electrochemical or uv detector. This method is also described in Halliwell et al. (1988).

Monitoring for transformation of deoxyribose to malonyldialdehyde by hydroxyl radicals

An alternative method for determining the level of "oxidant stress" (the relative level of oxygen centered radicals) in a living system is to monitor the presence of malondialdehyde (MDA). This method is known as the malondialdehyde test (or the deoxyribose assay).

Malondialdehyde arises from the decomposition of deoxyribose (or DNA) by hydroxyl radicals. The product of this decomposition, MDA, will react with thiobarbituric acid (TBA) to form a colored adduct which can be detected spectroscopically.

The general procedure is described in Halliwell et al. (1988). Additional modifications to the procedure of Halliwell et al. (describing the performance of this assay in parasitized erythrocytes) are described by Marva et al. (1988). Briefly, cells are cultured in the presence or absence of the suspected oxidant drug. At various times of incubation, cells are harvested by centrifugation and resuspended in 0.5 ml to 1ml of 100mM trichloroacetic acid (TCA) containing 100 mM sodium arsenite. The precipitable material is removed by centrifugation at 980g and 0.9 ml of the supernatant is incubated with 0.3 ml of 59mM thiobarbituric acid for 25 min at 100°C. The absorbance is determined according to the formula OD_{532}-OD_{600} x 156 and related to the number of cells or erythrocytes in the original suspension. Additional sensitivity can be accomplished by taking advantage of the fact that the adduct is fluorescent and can be detected by standard fluorometry with an excitation wavelength of 360nm and emission wavelength of 440nm.

EXAMPLE 3: Therapeutic formulations

The treatment of infectious diseases according to the present invention would be achieved by the administration of a formula 1 compound to a patient in combination with an oxidant agent. The formula 1 compound and the oxidant agent may be combined in a preparation prior to administration, or, they may be administered sequentially (preferably within 24 hours of each other). The formula 1 compound (either alone or in combination with an oxidant agent) will preferably be formulated with a pharmaceutically acceptable excipient prior to administration.

The formula 1 compound will be administered in a therapeutically effective dosage of 20-2,000 mg/kg/day and the oxidant agent will be administered in a therapeutically effective dosage of 0.1-2,000 mg/kg/day. One of skill in the art will recognize that the dosage chosen will depend on the particular formula 1 compound chosen and the particular oxidant agent chosen, as well as the usual clinical factors (age of patient, severity of infection, whether other medicines are being administered etc.) In a preferred embodiment of this invention, a "therapeutically effective dosage" is a dosage that results in complete remission of the disease. However, it will be appreciated that a therapeutically effective dosage also

encompasses dosages which slow or limit the further development of the disease process or which inhibit the growth of the disease pathogen.

Administration of the formula 1 compound and oxidant agent may be by any conventional means, including oral administration, intravenous injection and intramuscular injection.

By way of example, a patient suffering from malaria caused by *P. falciparum* could be treated by a single oral administration of a combined preparation of exifone and rufigallol. The preferred dosage range of these components would be 20-2,000 mg/kg (exifone) and 0.1-2,000 mg/kg (rufigallol).

By way of another example, a patient suffering from malaria caused by *P. falciparum* could be treated by a oral administration of exifone followed within 24 hours by oral administration of rufigallol, both in the same dosage range as described above.

Another aspect of the present invention is a kit containing comprising a formula 1 compound and an oxidant agent. Such a kit may be used to kill or inhibit the growth of *P. falciparum* in vitro, as well as providing a convenient package for use in administering the compounds to patients suffering from an infectious disease.

All publications and published patent documents cited in this specification are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

This invention has been detailed both by example and by direct description. It should be apparent that one having ordinary skill in this art would be able to surmise equivalents to the invention as described in the claims which follow but which would be within the spirit of the description above. Those equivalents are to be included within the scope of this invention.

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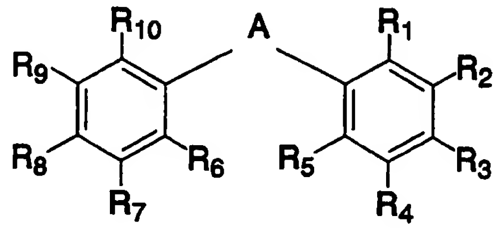
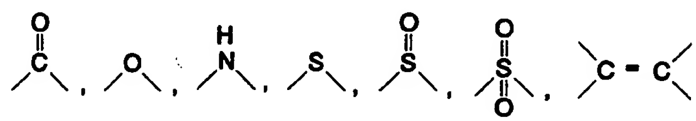
WHAT IS CLAIMED IS:

1. A composition comprising an oxidant agent and a compound having the formula:

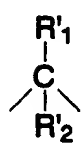


wherein:

A is selected from the group consisting of



 wherein R is H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine, and

 wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine or haloalkylamine;

wherein R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, OAc, OMe, NH_2, SO_3^-, N_3, alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl;

and wherein at least one of R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H.

2. The composition of claim 1 wherein the compound is 2,3,4,3',4',5' hexahydroxybenzophenone.

3. The composition of claim 1 wherein the compound is 2,3,4,2',3',4' hexahydroxybenzophenone.

4. The composition of claim 1 wherein the oxidant agent is rufigallol.

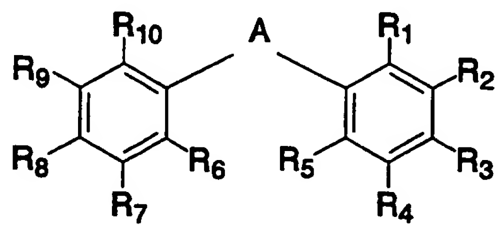
5. The composition of claim 1 wherein the oxidant agent is ascorbic acid.

6. A composition for the treatment of malaria comprising rufigallol.

7. The composition of claim 5 wherein the composition further comprises 2,3,4,3',4',5' hexahydroxybenzophenone.

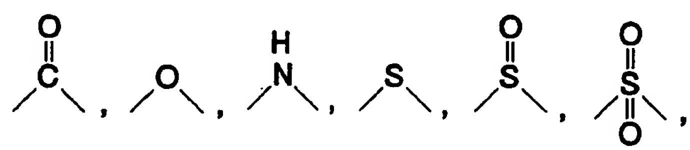
8. The composition of claim 5 wherein the composition further comprises 2,3,4,2',3',4' hexahydroxybenzophenone.

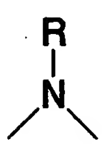
9. A kit comprising a compound having the formula

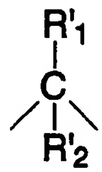


wherein:

A is selected from the group consisting of



 wherein R is H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine, and



wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine or haloalkylamine;

wherein R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, OAc, OMe, NH_2, SO_3^-, N_3, alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl;

and wherein at least one of R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H; and

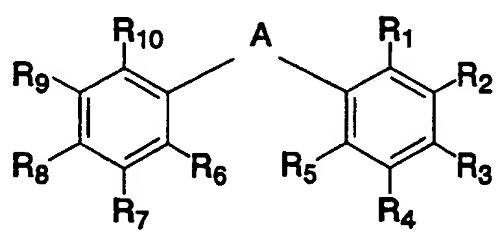
an oxidant agent, wherein the oxidant agent and the compound form a synergistic anti-pathogenic combination when administered to an animal suffering from an infectious disease caused by a pathogen.

10. A kit according to claim 9 wherein the compound is 2,3,4,3',4',5' hexahydroxybenzophenone.

11. A kit according to claim 9 wherein the compound is 2,3,4,2',3',4' hexahydroxybenzophenone.

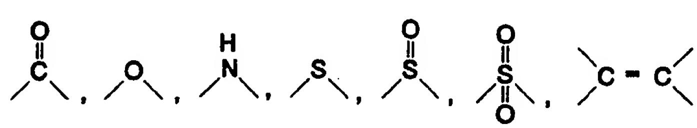
12. A kit according to claim 9 wherein the oxidant agent is an oxidant drug.

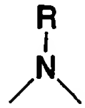
13. A preparation for treating an infectious disease comprising the compound



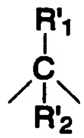
wherein:

A is selected from the group consisting of





wherein R is H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine, and



wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine or haloalkylamine;

wherein R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, OAc, OMe, NH_2, SO_3^-, N_3, alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl;

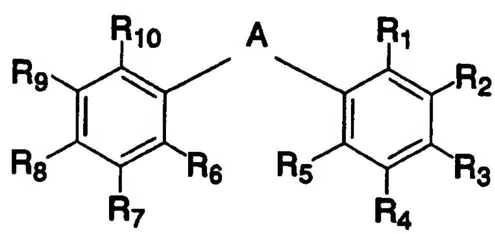
and wherein at least one of R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H;

and wherein the preparation is administered to a patient suffering from an infectious disease simultaneously or sequentially with an oxidant agent and wherein the compound acts synergistically with the oxidant agent.

14. The preparation of claim 13 wherein the infectious disease is a parasitic disease.

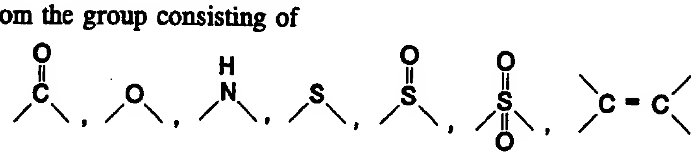
15. The preparation of claim 14 wherein the infectious disease is malaria.

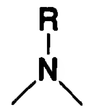
16. A method of potentiating a therapeutic efficacy of an oxidant agent comprising combining the oxidant agent with a compound having the formula:



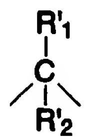
wherein:

A is selected from the group consisting of





wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine or haloalkylamine;



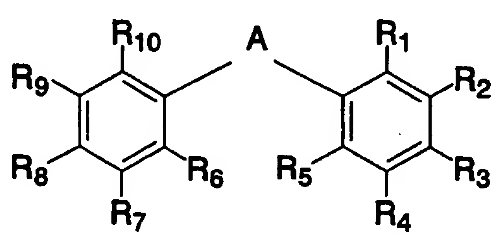
wherein R is H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine, and

wherein R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, OAc, OMe, NH_2, SO_3^-, N_3, alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl;

and wherein at least one of R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H.

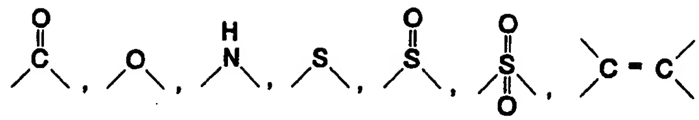
17. The method of claim 16 wherein the compound is 2,3,4,3'4',5' hexahydroxybenzophenone.

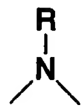
18. A method of treating a patient having an infectious disease caused by a pathogen, comprising administering to the patient, sequentially or simultaneously, a first compound and a second compound, the first compound being an oxidant agent and the second compound having the formula:



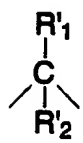
wherein:

A is selected from the group consisting of





wherein R is H, OH, alkyl, haloalkyl, alkylamine, or haloalkylamine, and



wherein R'_1 and R'_2 are independently either H, OH, alkyl, haloalkyl, alkylamine or haloalkylamine;

wherein R_1-R_{10} are independently selected from the group consisting of OH, H, halogen, OAc, OMe, NH_2, SO_3^-, N_3, alkyl, alkylamine, haloalkyl, aminoalkylether and $COOR_x$ where R_x is H or alkyl;

and wherein at least one of R_1, R_5, R_6 and R_{10} is OH, NH_2, NO_2 or OAc and at least one other of R_1, R_5, R_6 and R_{10} is H;

and wherein the first and second compounds are administered in a therapeutically effective dosage.

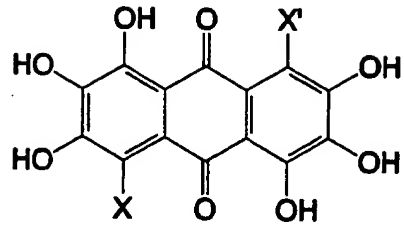
19. The method of claim 18 wherein the oxidant compound is an oxidant drug used to treat the infectious disease.

20. The method of claim 18 wherein the second compound is exifone or isofone.

21. The method of claim 20 wherein the oxidant agent is selected from the group consisting of ascorbic acid, rufigallol, artemisinin, doxorubicin and metronidazole.

22. The method of claim 18 wherein the infectious disease is malaria.

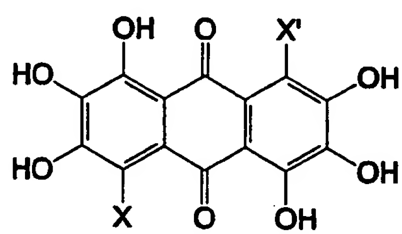
23. A composition for the treatment of malaria comprising a compound having a formula:



wherein X and X' are the same or different and are independently selected from the group consisting of alkyl, haloalkyl, diaminoalkyl, haloaminoalkyl, amino, nitro and azido.

24. A composition according to claim 23 wherein the compound is selected from the group consisting of 1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone, 1,2,3,5,6,7 hexaacetoxy-9,10-anthraquinone and 2,3,6,7-tetrahydroxy-9,10-anthraquinone.

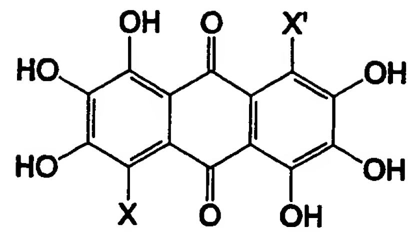
25. A method of treating a patient having malaria, comprising administering to the patient a therapeutically effective dose of a compound having a formula:



wherein X and X' are the same or different and are independently selected from the group consisting of alkyl, haloalkyl, diaminoalkyl, haloaminoalkyl, amino, nitro and azido.

26. The method of claim 25 wherein the compound is selected from the group consisting of 1,2,3,5,6,7,-hexahydroxy-9,10-anthraquinone, 1,2,3,5,6,7 hexaacetoxy-9,10-anthraquinone and 2,3,6,7-tetrahydroxy-9,10-anthraquinone.

27. A pharmaceutical composition comprising a compound having a formula:



wherein X and X' are the same or different and are independently selected from the group consisting of alkyl, haloalkyl, diaminoalkyl, haloaminoalkyl, amino, nitro and azido; and a pharmaceutical excipient.

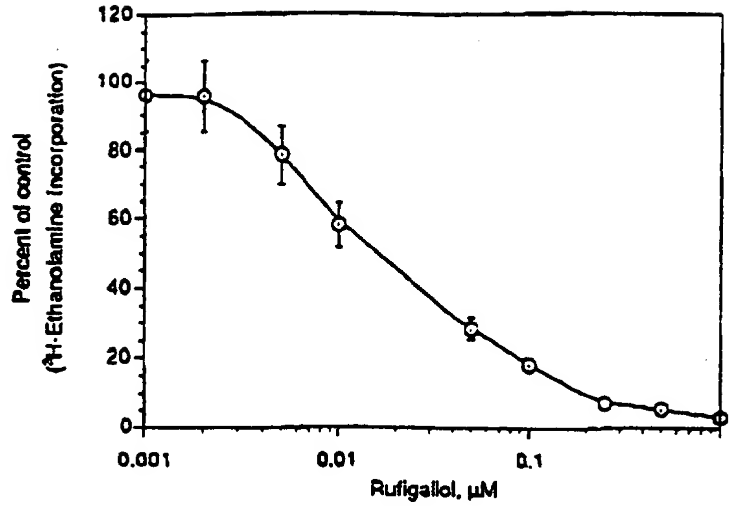


FIG. 1

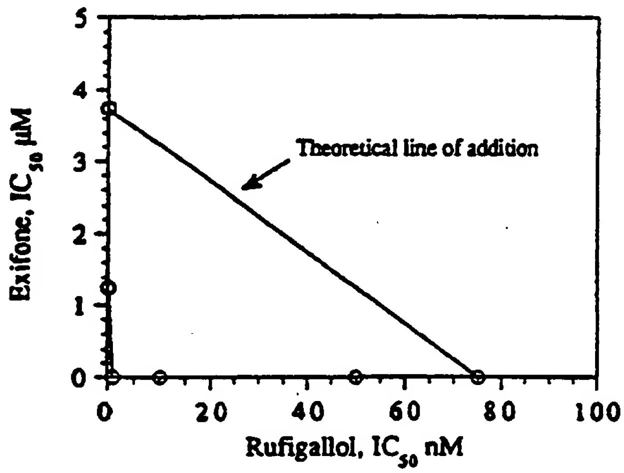


FIG. 2

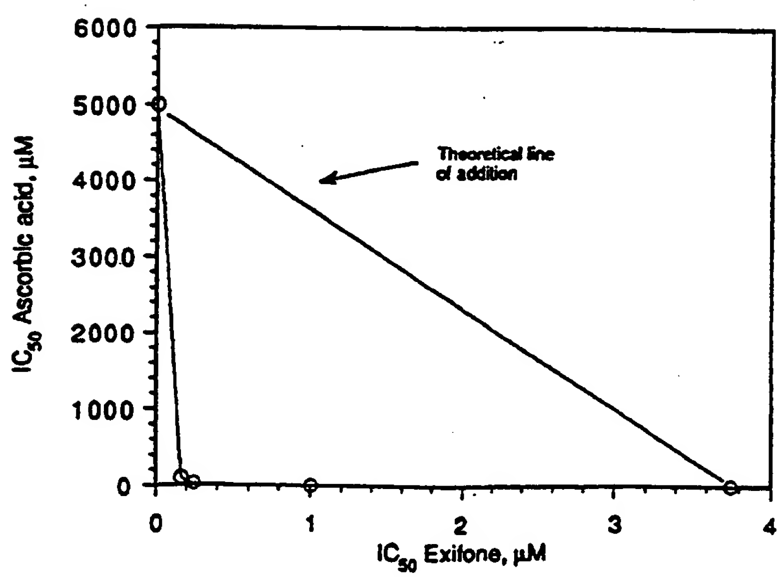


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13672

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/12, 31/135, 31/095, 31/10
US CL : 514/658, 680, 681, 706, 708, 709
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/658, 680, 681, 706, 708, 709

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,670,265 A (SYDISKIS ET AL) 02 June 1987, see entire document.	1-15
Y	US 3,050,440 A (RICHTER) 21 August 1962, see entire document.	1-21
Y	US 4,311,710 A (CLINTON ET AL) 19 January 1982, see entire document.	22
Y	US 3,947,594 A (RANDALL) 30 March 1976, see entire document.	23-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 DECEMBER 1996	16 DEC 1996

Name and mailing address of the ISA/US	Authorized officer
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